

From: Gabel, Gailene
Sent: Tuesday, January 07, 2003 10:57 AM
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- 1) Born and Cross, J. Physiol., 1963, 166:29-30.
- 2) Kitakaze et al., Endogenous adenosine inhibits platelet aggregation during myocardial ischemia in dogs. CIRCULATION RESEARCH, (1991 Nov) 69 (5) 1402-8.
- 3) Soederbaeck et al., Anti-aggregatory effects of physiological concentrations of adenosine in human whole blood as assessed by filragnetometry. Clinical Science (1991), 81(5), 691-4.
- 4) Singh et al., Effect of adenosine and inosine administration on platelet function in rabbits. Indian Journal of Physiology and Pharmacology (1990),34(1), 63-4.
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- 7) Born et al., Role of the competition in inhibition of platelet aggregation by adenosine. ACTA MEDICA SCANDINAVICA. SUPPLEMENTUM, (1971) 525 173-4.

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DIFFERENTIAL INHIBITION BY ADENOSINE OR BY PROSTAGLANDIN E₁ OF HUMAN PLATELET AGGREGATION INDUCED BY ADENOSINE 5'-O-(1-THIODIPHOSPHATE) AND ADENOSINE 5'-O-(2-THIODIPHOSPHATE)

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Adenosine 5'-diphosphate (ADP) induces human platelet aggregation and inhibits stimulated adenylate cyclase. Adenosine 5'-O-(1-thiodiphosphate) (ADP- α -S) and adenosine 5'-O-(2-thiodiphosphate) (ADP- β -S) act at the ADP receptor and achieve the same maximal rate of human platelet aggregation as each other. Adenosine and prostaglandin E₁, which noncompetitively inhibit ADP-induced aggregation by stimulating adenylate cyclase, inhibit aggregation induced by ADP- α -S more than aggregation induced by ADP- β -S. ADP- α -S, unlike ADP- β -S and ADP itself, does not inhibit stimulated adenylate cyclase. This suggests that the inhibition of stimulated adenylate cyclase by ADP, although not a cause of aggregation, may be of physiological importance in reducing the effects of endogenous agents such as adenosine and prostaglandins (for example, prostacyclin) to which the platelet may be exposed.

Introduction Adenosine 5'-diphosphate (ADP) induces human platelet aggregation (Born, 1962), and also causes noncompetitive inhibition of stimulated platelet adenylate cyclase (Haslam & Rosson, 1975). Agents which stimulate adenylate cyclase, such as prostaglandin E₁ (PGE₁), prostacyclin and adenosine, noncompetitively inhibit aggregation induced by ADP and other aggregating agents (Mills & Smith, 1971; Haslam & Rosson, 1975; Haslam, Davidson, Davies, Lynham & McClenaghan, 1978). Since an increase in levels of platelet adenosine 3', 5'-cyclic monophosphate (cyclic AMP) inhibits platelet aggregation, it has been suggested that the inhibition of adenylate cyclase might be a mechanism by which aggregation is induced (Salzman, 1972). However, some aggregating agents, such as vasopressin, do not inhibit stimulated adenylate cyclase (Haslam & Rosson, 1975), and intracellular inhibition of adenylate cyclase does not induce or potentiate aggregation (Haslam, Davidson & Desjardins, 1978). In addition, adenosine 5'-O-(1-thiodiphosphate) (ADP- α -S) does not inhibit stimulated adenylate cyclase (Cusack & Hourani, 1981b), but induces human platelet aggregation to the same extent as adenosine 5'-O-(2-thiodiphosphate) (ADP- β -S), which does inhibit stimulated adenylate cyclase (Cusack & Hourani, 1981a).

Although the inhibition by ADP of adenylate cyclase cannot be the cause of aggregation, it could limit the inhibitory effects of agents such as PGE₁ and adenosine which activate adenylate cyclase. The observation that PGE₁ and adenosine are more powerful inhibitors of aggregation induced by vasopressin than by ADP (Haslam & Rosson, 1972), is consistent with this suggestion, although ADP and vasopressin do not act at the same receptor (Macfarlane & Mills, 1975). The differential effects on adenylate cyclase of ADP- α -S and ADP- β -S, which both act at the ADP receptor (Cusack & Hourani, 1981a,b), provides the first opportunity to investigate whether inhibition of adenylate cyclase by ADP receptor agonists can limit the effects on aggregation of inhibitors such as adenosine and PGE₁.

Methods Human platelet-rich plasma (PRP) was obtained by centrifuging citrated venous blood at 260 g for 20 min and collecting the supernatant. Aggregation was quantified photometrically with a Born-Michal Mark IV aggregometer as the maximal rate of change in light transmission (expressed as arbitrary units/min) (Born, 1962; Michal & Born, 1971) through a sample (0.5 ml) of stirred PRP at 37°C on addition of an aggregating agent. Solutions (10 μ l) of adenosine (final concentration 10 μ M), PGE₁ (final concentration 40 nM) or saline were preincubated with the PRP for 30 s at 37°C before addition of a solution (10 μ l) of ADP- α -S or ADP- β -S.

Adenosine was obtained from Sigma London. PGE₁ was a generous gift from Dr J. Pike of the Upjohn Company in Kalamazoo, Michigan. Adenosine 5'-monophosphorothioate (AMPS) and ADP- β -S were obtained from Boehringer Mannheim and purified by ion exchange chromatography before use. ADP- α -S was synthesized by phosphorylation of AMPS as described by Eckstein & Goody (1976). The diastereoisomers of ADP- α -S (Eckstein & Goody, 1976; Cusack & Hourani, 1981b) were not separated.

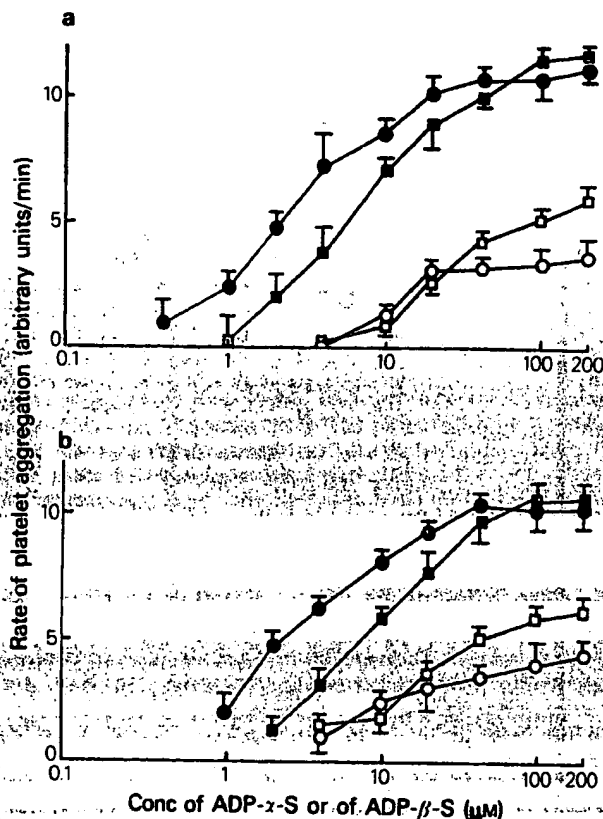


Figure 1 Inhibition by (a) adenosine (10 μM) or (b) prostaglandin E (PGE_1 40 nM) of human platelet aggregation induced by adenosine 5'-O-(1-thiodiphosphate) (ADP- α -S) and adenosine 5'-O-(2-thiodiphosphate) (ADP- β -S). Platelet-rich plasma (PRP) was preincubated for 30 s at 37°C with saline (closed symbols) or an inhibitor (open symbols) before addition of ADP- α -S (\bullet , \circ) or ADP- β -S (\blacksquare , \square). Each point is the mean of at least three observations. Vertical bars show standard deviations.

Results Log dose-response curves to ADP- α -S and ADP- β -S were parallel and reached the same maximum, and ADP- α -S was more potent than ADP- β -S (Figure 1a and b). Adenosine (10 μM) (Figure 1a) or PGE_1 (40 nM) (Figure 1b) noncompetitively inhibited aggregation induced by ADP- α -S and ADP- β -S. In the presence of adenosine (Figure 1a) or PGE_1 (Figure 1b), aggregation induced by ADP- α -S was

inhibited more than aggregation induced by ADP- β -S.

Discussion ADP- α -S and ADP- β -S are partial agonists each with an intrinsic activity of about 0.75 for aggregation at the platelet ADP receptor (Cusack & Hourani, 1981a,b), and the results presented here, where they are directly compared with each other, show that they do reach the same maximal rate of aggregation. Whereas ADP- β -S (like ADP) inhibits stimulated adenylate cyclase (Cusack & Hourani, 1981b), ADP- α -S does not, although it does antagonize competitively this action of ADP (Cusack & Hourani, 1981b).

As a consequence of having a chiral α phosphate, ADP- α -S exists as a pair of S_P and R_P diastereoisomers (Eckstein & Goody, 1976). Each diastereoisomer induces human platelet aggregation to the same extent, and neither inhibits stimulated adenylate cyclase. The S_P diastereoisomer is about 5 times more potent than the R_P diastereoisomer, both as an aggregating agent and as an antagonist of the action of ADP on stimulated adenylate cyclase (Cusack & Hourani, 1981b). Since each diastereoisomer has essentially the same action on human platelets, it was not necessary in this study to separate them.

Our results here show that although ADP- α -S (the nett effect of the unseparated diastereoisomers) was a more potent aggregating agent than ADP- β -S, ADP- α -S was inhibited more than ADP- β -S both by adenosine (Figure 1a) and by PGE_1 (Figure 1b). The inhibition of adenylate cyclase by an ADP receptor agonist, ADP- β -S, does therefore appear to reduce the inhibition of aggregation caused by agents which stimulate adenylate cyclase. The physiological significance of the inhibition of adenylate cyclase by ADP itself may therefore be to limit the effects of endogenous adenosine or prostacyclin to which platelets may be exposed.

We thank the Medical Research Council (G8111856) and the British Heart Foundation (81/32) for support for N.J.C., Sandoz Products Limited for support for S.M.O.H., the Fritz Thyssen Stiftung for an equipment grant and Professor G.V.R. Born, F.R.C.P., F.R.S. for encouragement. Blood was taken by Dr C.G. Fenn of this department. Correspondence to S.M.O.H. please.

References

- BORN, G.V.R. (1962). Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature, Lond.*, 194, 927-929.
- CUSACK, N.J. & HOURANI, S.M.O. (1981a). Partial agonist behaviour of adenosine 5'-O-(2-thiodiphosphate) on human platelets. *Br. J. Pharmac.*, 73, 405-408.
- CUSACK, N.J. & HOURANI, S.M.O. (1981b). Effects of R_P and S_P diastereoisomers of adenosine 5'-O-(1-thiodiphosphate) on human platelets. *Br. J. Pharmac.*, 73, 409-412.
- ECKSTEIN, F. & GOODY, R.S. (1976). Synthesis and properties of diastereoisomers of adenosine 5'-(O-1-

- thiotriphosphate) and adenosine 5'-(O-2-thiotriphosphate). *Biochemistry*, **15**, 1685-1699.
- HASLAM, R.J., DAVIDSON, M.M.L., DAVIES, T., LYNHAM, J.A. & McCLENAGHAN, M.D. (1978). Regulation of blood platelet function by cyclic nucleotides. *Adv. Cyclic Nucleotide Res.*, **9**, 533-552.
- HASLAM, R.J., DAVIDSON, M.M.L. & DESJARDINS, J.V. (1978). Inhibition of adenylate cyclase by adenosine analogues in preparations of broken and intact human platelets. Evidence for the unidirectional control of platelet function by cyclic AMP. *Biochem. J.*, **176**, 83-95.
- HASLAM, R.J. & ROSSON, G.M. (1972). Aggregation of human blood platelets by vasopressin. *Am. J. Physiol.*, **223**, 958-967.
- HASLAM, R.J. & ROSSON, G.M. (1975). Effects of adenosine on levels of adenosine cyclic 3',5'-monophosphate in human blood platelets in relation to adenosine incorporation and platelet aggregation. *Mol. Pharmacol.*, **11**, 528-544.
- MACFARLANE, D.E. & MILLS, D.C.B. (1975). The effects of ATP on platelets: evidence against the central role of released ADP in primary aggregation. *Blood*, **46**, 309-320.
- MICHAL, F. & BORN, G.V.R. (1971). Effect of the rapid shape change of platelets on the transmission and scattering of light through plasma. *Nature, Lond.*, **231**, 220-222.
- MILLS, D.C.B. & SMITH, J.B. (1971). The influence on platelet aggregation of drugs that affect the accumulation of adenosine 3':5'-cyclic monophosphate in platelets. *Biochem. J.*, **121**, 185-196.
- SALZMAN, E.W. (1972). Cyclic AMP and platelet function. *New Engl. J. Med.*, **286**, 358-363.

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Table 2 Effect of Inhibitor Combinations on ^{14}C -Adenosine Uptake by Human Platelets

(A) Inosine	5	Dipyridamole ($\text{M} \times 10^{-8}$)			None
		24.5	1.25		
2.5×10^{-4}	67.2 (64)*	54.7 (60)	48.3 (57.2)	50.3	
5×10^{-5}	62 (46)	34 (37)	29 (23)	30	
Buffer	42†	27.6	8	0	
(B) Hypoxanthine					
2.5×10^{-4}	60 (49)	37 (36)	28 (18)	16	
5×10^{-5}	60 (49)	41 (32)	30 (19)	3.5	
Buffer	42	29	15	0	
(C) Adenine					
2.5×10^{-4}	71.5 (60)	44.5 (44)	39 (34)	25	
5×10^{-5}	66 (60)	40 (37)	33 (17)	19	
Buffer	47.5	34	10	0	
(D) Uric acid					
3.8×10^{-5}	57.5 (56)	34 (27)	21 (15)	4	
Buffer	46	26	13	0	

Platelets in PRP were incubated with the first agent (buffer, inosine or dipyridamole) for 5 min, and then the second agent was added and incubation continued for a further 5 min. ^{14}C -Adenosine ($0.05 \mu\text{Ci/mol}$) was then added to the mixture and the incubation was either stopped or continued for 20 min. The reaction was carried out at 37°C and stopped by the addition of adenosine 5×10^{-4} M final concentration¹². The mixture was then treated as described in Table 1.

* Results in parentheses are where dipyridamole was added first.

† The counts per min associated with platelets treated only with buffer were arbitrarily called zero percentage inhibition.

uptake more than either agent alone. With the low concentration of inosine in combination with the highest concentration of dipyridamole the inhibition depended on the order in which the agents were added (Table 2A). With hypoxanthine (2.5×10^{-4} or 5×10^{-5} M) plus dipyridamole, the decrease in adenosine uptake did not depend on the hypoxanthine concentration but again depended on the order in which the agents were added (Table 2B).

Combinations of adenine (2.5×10^{-4} or 5×10^{-5} M) with dipyridamole increased the inhibition of uptake but did not depend on adenine concentration. As with hypoxanthine, inhibition depended on the order in which the agents were added (Table 2C). Uric acid (3.8×10^{-5} M) decreased the inhibition of incorporation of adenosine into platelets slightly and only with the highest concentration of dipyridamole (Table 2D). To find whether the increased inhibition of adenosine uptake produced by inosine or hypoxanthine was due to their breakdown to uric acid, we determined the concentration of uric acid in plasma containing inosine and hypoxanthine. We detected no uric acid with the concentrations of inosine and hypoxanthine used.

Inosine, hypoxanthine and dipyridamole, singly or in combination, therefore seem to inhibit adenosine uptake directly at the platelet membrane.

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- Born, G. V. R., and Cross, M. J., *J. Physiol.*, **168**, 178 (1963).
- Born, G. V. R., Honour, A. J., and Mitchell, J. R. A., *Nature*, **202**, 761 (1964).
- Born, G. V. R., *Nature*, **202**, 95 (1964).
- Constantine, J. W., *Nature*, **205**, 1075 (1965).
- Skoza, L., Zucker, M. B., Jerushalmy, Z., and Grant, R., *Thromb. Diath. Haemorrh.*, **18**, 7:3 (1967).
- Ardlie, N. G., Packham, M. A., and Mustard, J. F., *Amer. J. Physiol.*, **217**, 1009 (1969).
- Rozenberg, M. R., and Holmsen, H., *Biochim. Biophys. Acta*, **155**, 342 (1968).
- Born, G. V. R., and Mills, D. C. B., *J. Physiol.*, **202**, 41 (1969).
- Markwardt, F., Barthel, W., Glusa, E., and Hoffman, A., *Arch. Pharmacol. Exp. Pathol.*, **257**, 420 (1967).
- Caen, J. P., Jenkins, C. S. P., Michel, H., and Bellanger, R., *Nature (New Biology)*, **239**, 211 (1972).
- Pinkhas, J., Chivot, J. J., Michel, H., and Caen, J. P., *Europ. J. Clin. Biol. Res.*, **15**, 984 (1970).
- Vainer, H. (Plenum, New York, in the press).
- Caen, J. P., and Michel, H., *Second Congr. Intern. Soc. on Thrombosis and Haemostasis, Oslo, Abstr.*, **184** (1971).
- Michel, H., thesis, Université de Paris (1972).

Adenosine Inhibition of Human Platelet Aggregation by ADP

We have shown that in human platelet-rich plasma, inosine is a concentration-dependent inhibitor of adenosine incorporation into platelets^{1,2}, and at high concentrations inosine inhibits adenosine decomposition³. This prompted us to investigate the effect of inosine and other adenosine decomposition products on aggregation of human platelets *in vivo* by ADP.

Human citrated platelet-rich plasma was incubated at 37°C with either inosine or hypoxanthine at a final concentration of 10^{-3} M or uric acid at 3.8×10^{-5} M for various times before ADP (5×10^{-6} M) was added. The effects on aggregation were followed by the method of Born⁴.

At high concentration (10^{-3} M), inosine inhibits platelet aggregation by ADP¹. Lower concentrations also inhibit; this inhibition is maintained for 40 to 90 min, by which time the inhibitory effect of adenosine alone had disappeared (Fig. 1). Hypoxanthine (10^{-3} M) inhibited aggregation by ADP slightly and only after 90 min (Fig. 1). Inosine plus hypoxanthine inhibited induced aggregation much more than either substance did alone. Inhibition was observed after 5 min and

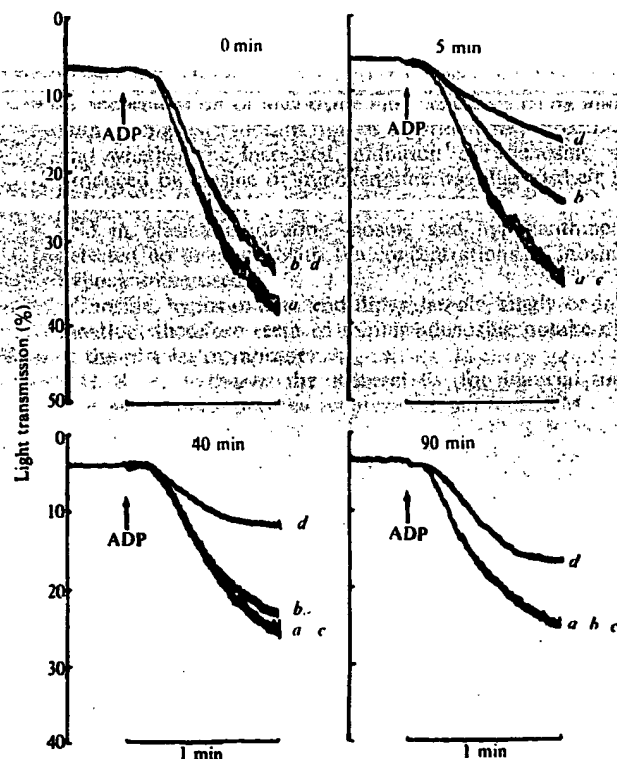


Fig. 1 Effect of inosine and hypoxanthine alone and in combination on ADP-induced platelet aggregation in human citrated PRP. a, Buffer; b, adenine (10^{-3} M); c, inosine (10^{-3} M); d, adenine+inosine. Time of incubation before addition of ADP (5×10^{-6} M) given at top of graphs.

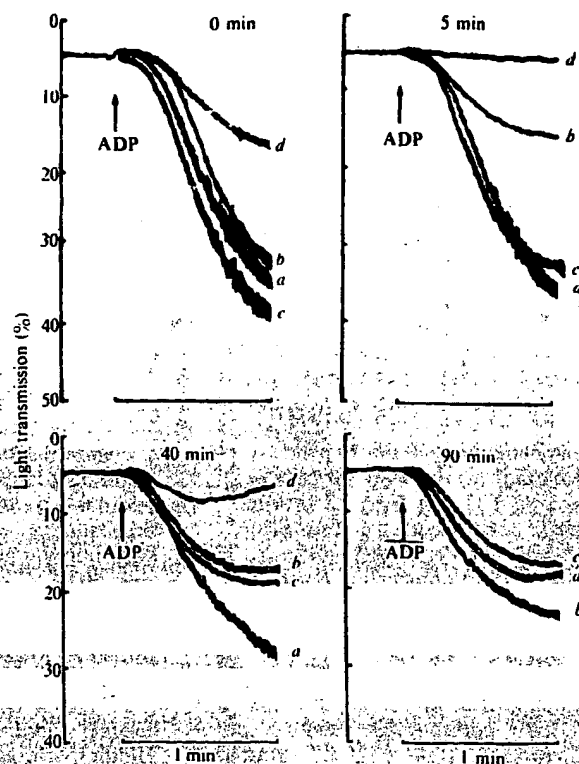


Fig. 2 Effect of inosine on adenosine inhibition of ADP-induced platelet aggregation in human citrated PRP. *a*, Buffer; *b*, adenosine (2.5×10^{-6} M); *c*, hypoxanthine (10^{-3} M); *d*, adenosine + hypoxanthine. Time of incubation before addition of ADP (5×10^{-6} M) given at top of graphs.

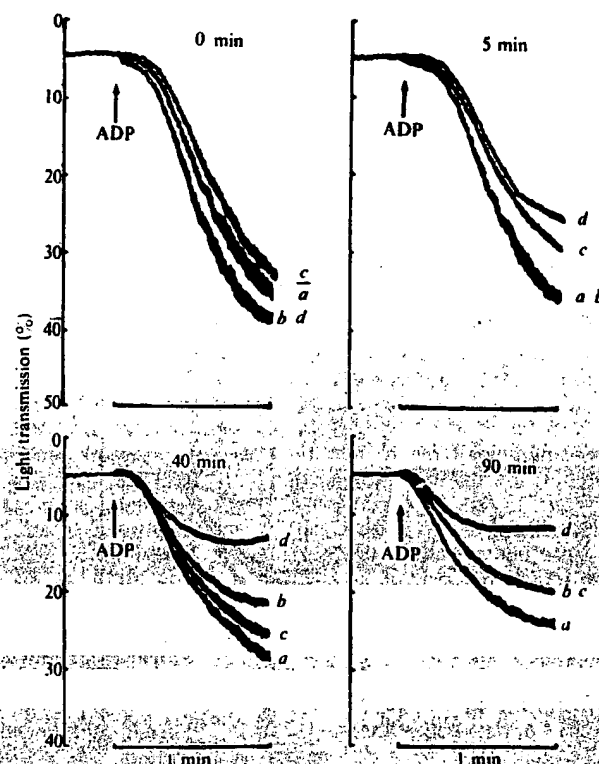


Fig. 3 Effect of hypoxanthine on adenosine inhibition of ADP-induced platelet aggregation in human citrated PRP. *a*, Buffer; *b*, hypoxanthine (10^{-3} M); *c*, inosine (10^{-3} M); *d*, hypoxanthine + inosine. Time of incubation before addition of ADP (5×10^{-6} M) given at top of graphs.

maintained for at least 90 min (Fig. 1). Uric acid did not influence aggregation of human platelets by ADP.

It is difficult to explain how these agents affect aggregation but several possibilities may be considered. Plasma inosine and hypoxanthine may equilibrate to give a mixture of both agents and perhaps generate adenosine. These compounds may exist in equilibrium in a complex concentration dependent form responsible for the observed inhibition. The proposed equilibrium could explain why hypoxanthine does not immediately inhibit ADP-induced aggregation; the conversion from hypoxanthine to inosine may be slow and a long incubation time is necessary. Fig. 1 shows that when platelet-rich plasma is incubated for 90 min with either inosine or hypoxanthine alone, the aggregation tracings are identical.

Alternatively, inosine and hypoxanthine may inhibit some platelet function essential for aggregation. The accumulation of inosine and hypoxanthine in plasma may partly explain the loss of response to ADP of platelets during storage.

Human citrated platelet-rich plasma was incubated with inosine or hypoxanthine at 10^{-3} M and with adenosine at 2.5×10^{-6} M or 5×10^{-6} M for various times before addition of ADP at 5×10^{-6} M. The addition of inosine increased and prolonged inhibition by adenosine, and this inhibition disappeared slowly later (Fig. 2). The combination of hypoxanthine and adenosine produced immediate inhibition which became complete after 5 min, but disappeared slowly with further incubation. After 90 min, hypoxanthine by itself was as effective as in combination with adenosine, whereas the platelets preincubated with adenosine were more sensitive to ADP than controls after 90 min (Fig. 3).

Inosine, hypoxanthine and adenosine all together gave an immediate inhibition and the inhibition was complete after 40 min. After 90 and 120 min, a little aggregation was restored (Fig. 4).

Inosine at the concentrations used strongly inhibits adenosine incorporation^{1,2} and adenosine deaminase activity (ADA).

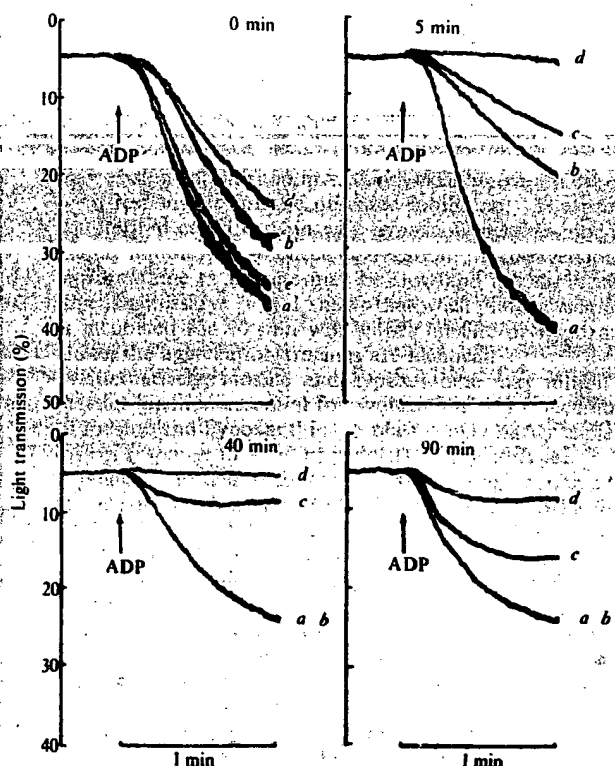


Fig. 4 Effect of a combination of inosine and hypoxanthine on adenosine inhibition of ADP-induced platelet aggregation in human citrated PRP. *a*, Buffer; *b*, adenosine (2.5×10^{-6} M); *c*, adenosine (2.5×10^{-6} M) + inosine (10^{-3} M); *d*, adenosine (2.5×10^{-6} M) + inosine (10^{-3} M) + hypoxanthine (10^{-3} M). Time of incubation before addition of ADP (5×10^{-6} M) given at top of graphs.

Inosine therefore inhibits both systems for removing plasma adenosine. Adenosine ought therefore to remain in the plasma when inosine is present and inhibit aggregation for longer.

Hypoxanthine does not inhibit adenosine incorporation into human platelets^{1,2} nor does it influence the action of plasma ADA at the concentrations we used. A combination of hypoxanthine and adenosine may inhibit aggregations effectively because adenosine, inosine and hypoxanthine equilibrate to form a mixture that inhibits aggregation by ADP. Any inosine produced will inhibit the incorporation of adenosine and thus reduce the loss of adenosine. After 90 min incubation of platelet-rich plasma with either hypoxanthine or hypoxanthine plus adenosine, we observed the same inhibition of aggregation by ADP. This may have been due to the equilibration of hypoxanthine or hypoxanthine plus adenosine in the same proportions.

Inosine inhibits the incorporation of adenosine into human platelets whereas hypoxanthine does not⁴. The effect of these agents on the conversion of adenosine into nucleotides is not known. It may be that, although the uptake of adenosine is not inhibited by hypoxanthine, it somehow inhibits the conversion of adenosine into nucleotides. If this were so, hypoxanthine might allow adenosine to remain in the membrane and so exert an inhibitory effect for a longer time. This suggestion is similar to Holmsen and Rozenberg's⁵ explanation how papaverine augments adenosine-inhibition of aggregation. They suggest that papaverine might retard the transport of adenosine across the platelet membrane.

On this basis, inosine and hypoxanthine in combination would be expected to potentiate further the inhibition by adenosine (Fig. 4). Our observations are compatible with the assumption of Holmsen and Day⁶ that the inhibitory concentration of adenosine is extremely small and not accessible to extracellular enzymes.

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- ³ Pinkhas, J., Chivot, J. J., Michel, H., and Caen, J. P., *Europ. J. Clin. Biol. Res.*, 15, 984 (1970).
- ⁴ Born, G. V. R., *Nature*, 194, 927 (1962).
- ⁵ Rozenberg, M. C., and Holmsen, H., *Biochim. Biophys. Acta*, 155, 342 (1968).
- ⁶ Holmsen, H., and Day, H. J., *Series Haematol.*, 4, 28 (1971).

Selective Uptake of 5-Hydroxytryptamine by Axonal Processes in *Helix pomatia*

THE neurotransmitter substances released from presynaptic terminals may be inactivated in different ways: (1) by metabolic conversion to inactive substances, (2) by diffusion away from the synaptic area, or (3) by cellular uptake from the region of the synaptic cleft; for example, by re-uptake into the presynaptic terminals¹.

5-Hydroxytryptamine (5-HT)—often referred to as serotonin—is very probably a neurotransmitter in the central nervous system of gastropod molluscs^{2,3}, and there is good evidence that this amine is also involved in mediating neurally induced cardio-acceleration^{4,5} and muscle relaxation⁶ in some bivalve molluscs.



Fig. 1 Electron microscope autoradiograph of part of the neuropile in a buccal ganglion of *H. pomatia* (see Fig. 2). Two obliquely sectioned axons are heavily labelled with ³H-5-HT. Such axons contain granular vesicles (see arrows in the magnified inset).

However, ganglia of gastropod molluscs do not seem to possess enzymes that inactivate 5-HT^{7,8}. Gerschenfeld and Stefani⁹ have suggested that diffusion plays an important role in terminating the action of 5-HT in molluscan nervous tissue, but it seems likely that a cellular uptake of 5-HT is also involved. This is because imipramine, which blocks the uptake of 5-HT by the snail central ganglia (our unpublished work), markedly potentiates serotonergic transmission in this animal⁹. There is also evidence that axons in the heart of *Aplysia* take up 5-HT¹⁰.

We have investigated the site of 5-HT uptake in the snail nervous system using autoradiographic techniques. The results obtained show that the amine is taken up in a highly selective manner by certain axon processes and presumed nerve endings some of which occur in regions thought to contain serotonergic synapses.

Two types of experiments were made with specimens of the snail *Helix pomatia*. In the first type, a cannula was inserted into the anterior aorta, branches of which supply the central ganglia¹¹, and then saline¹² containing 0.5–1.5 nM uniformly ³H-5-HT (specific activity 12 Ci/mmol) was perfused for 5–15 h before killing the animal. In the second experiments, the central ganglia were dissected from live animals, the outer connective-tissue layer removed, and the nervous tissue placed in small dishes of saline containing 1.25 nM uniformly labelled ³H-5-HT for 5–10 h at 18° C. In both sets of experiments, tissue was fixed in glutaraldehyde and osmium tetroxide solutions. For light microscope autoradiography, 10 μm paraffin sections were coated with 'Kodak AR.10' stripping film, exposed from 2 days to 2 weeks, and developed in 'Kodak D.19' developer. For electron microscope autoradiography, thin 'Araldite' sections were covered with a monolayer of 'Ilford L.4' emulsion by a loop technique¹³, exposed from 1 to 4 months, and subsequently developed in 'Kodak Mikrodol-X'.

Under the experimental conditions used, 5-HT was taken up selectively by some axons and presumed endings in the neuropile region of each central ganglion. Certain axons in the connective tissue sheath of the ganglia and in the peripheral musculature also accumulated 5-HT. A common feature of all the labelled axons was the presence of vesicles with electron-dense cores (Fig. 1). These vesicles have an average diameter of 100 nm and are morphologically identical to those which sequester 5-HT in the giant 5-HT-containing cell in the cerebral ganglion of the snail and closely related slug, *Limax maximus*¹⁴; similar dense-cored vesicles have been shown to contain 5-HT in the Retzius cell of the leech¹⁵. Because many of the labelled snail axons contained many vesicles in neuropile regions, they are probably terminal regions of axons close to nerve endings.

(FILE 'HOME' ENTERED AT 10:04:02 ON 07 JAN 2003)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, CAPLUS, USPATFULL' ENTERED AT
10:04:31 ON 07 JAN 2003

L1	1 S ADENOSINE (A) BIND? (A) (GLYCOPROTEIN? OR GPIIBIIIA)
L2	70 S ADENOSINE (3A) (ANTITHROMB? OR (INHIBIT? THROMB))
L3	578 S ADENOSINE (3A) (INHIBIT? (2A) PLATELET AGGREGAT?)
L4	8 S L2 AND L3
L5	7 DUP REM L4 (1 DUPLICATE REMOVED)
L6	44 DUP REM L2 (26 DUPLICATES REMOVED)
L7	419 S L3 AND ADENOSINE/AB
L8	203 S L3 AND ADENOSINE/TI
L9	107 DUP REM L8 (96 DUPLICATES REMOVED)

=>

Gabel, Gailene

To: STIC-ILL
Subject: 09/853,524

Please provide a copy of the ff. literature ASAP:

- 1) Born and Cross, J. Physiol., 1963, 166:29-30.
- 2) Kitakaze et al., Endogenous adenosine inhibits platelet aggregation during myocardial ischemia in dogs. CIRCULATION RESEARCH, (1991 Nov) 69 (5) 1402-8.
- 3) Soederbaeck et al., Anti-aggregatory effects of physiological concentrations of adenosine in human whole blood as assessed by filtragometry. Clinical Science (1991), 81(5), 691-4.
- 4) Singh et al., Effect of adenosine and inosine administration on platelet function in rabbits. Indian Journal of Physiology and Pharmacology (1990),34(1), 63-4.
- 5) Cusack et al., Differential inhibition by adenosine or by prostaglandin E1 of human platelet aggregation induced by adenosine 5'-O-(1-thiodiphosphate) and adenosine 5'-O-(2-thiodiphosphate). BRITISH JOURNAL OF PHARMACOLOGY, (1982 Feb) 75 (2) 257-9.
- 6) Caen et al., Adenosine inhibition of human platelet aggregation by ADP. NATURE. NEW BIOLOGY, (1972 Oct 18) 239 (94) 211-3.
- 7) Born et al., Role of the competition in inhibition of platelet aggregation by adenosine. ACTA MEDICA SCANDINAVICA. SUPPLEMENTUM, (1971) 525 173-4.

L1 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:488244 CAPLUS

DOCUMENT NUMBER: 137:41748

TITLE: Adenosine as antithrombotic

INVENTOR(S): Chang, Su-Chen; Hsu, Li-Wei

PATENT ASSIGNEE(S): Taiwan

SOURCE: U.S. Pat. Appl. Publ., 19 pp., Cont.-in-part of U.S.
Ser. No. 708,306.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002082241	A1	20020627	US 2001-853524	20010510
CN 1352945	A	20020612	CN 2001-120742	20010528
CN 1353003	A	20020612	CN 2001-132691	20010904
JP 2002212080	A2	20020731	JP 2001-340593	20011106
PRIORITY APPLN. INFO.:			US 2000-708306	A2 20001107
			US 2001-853524	A 20010510

AB The present invention discloses a specific binding of adenosine to a platelet membrane receptor protein gpIIb/IIIa, and relates to the novel use of adenosine for inhibiting platelet aggregation and thrombosis. The present invention discloses that adenosine is useful as an antithrombotic. An active ingredient obtained from the fractions of an ext. of Carthamus tinctorius L specifically bound to gpIIb/IIIa. The platelet aggregation-inhibiting activity and antithrombic activity of adenosine were detd. The active ingredient was then identified as adenosine.

ST adenosine antithrombotic; **gpIIbIIIa binding**
adenosine Carthamus tinctorius ext

L5 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1987:534633 CAPLUS

DOCUMENT NUMBER: 107:134633

TITLE: Preparation of 2'-deoxy-2'-(ethylthio)
**adenosine as a blood platelet
aggregation inhibitor**

INVENTOR(S): Atami, Toshio; Aono, Shunji

PATENT ASSIGNEE(S): Sumitomo Pharmaceuticals Co., Ltd., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 5 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 62099394	A2	19870508	JP 1985-240265	19851025
PRIORITY APPLN. INFO.:			JP 1985-240265	19851025
OTHER SOURCE(S):		CASREACT 107:134633		

AB The title nucleoside (I), which inhibits blood platelet aggregation and is useful as an antithrombotic, was prepd. by reaction of D-arabinofuranosyladenine deriv. II [R = CF₃SO₂, R₁R₂ = (Me₂CH)₂SiOSi(CHMe₂)₂] (III) with EtOM (M = metal) followed by deprotection with Bu₄NE. Protection of II (R = R₁ = R₂ = H) with (Me₂CH)₂SiClOSiCl(CHMe₂)₂ in pyridine followed by treatment with CF₃SO₂Cl in CH₂Cl₂ contg. 4-(dimethylamino)pyridine at 0.degree. gave III which reacted with EtSNa in DMF to give I after deprotection. I at 30 .mu.g/mL inhibited by 94% blood platelet aggregation induced by adenosine 5-diphosphate in rabbit plasma.

TI Preparation of 2'-deoxy-2'-(ethylthio)**adenosine as a blood
platelet aggregation inhibitor**

ST deoxyethylthioadenosine prepn blood platelet aggregation inhibitor;
antithrombotic deoxyethylthioadenosine prepn; **adenosine**
deoxyethylthio prepn **antithrombotic**

IT Anticoagulants and **Antithrombotics**
Blood **platelet aggregation inhibitors**
(deoxy(ethylthio)**adenosine**)

L5 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1987:534633 CAPLUS

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TI Preparation of 2'-deoxy-2'-(ethylthio)**adenosine as a blood platelet aggregation inhibitor**

ST deoxyethylthioadenosine prepn blood platelet aggregation inhibitor; antithrombotic deoxyethylthioadenosine prepn; **adenosine** deoxyethylthio prepn **antithrombotic**

IT Anticoagulants and **Antithrombotics**
Blood **platelet aggregation inhibitors**
(deoxy(ethylthio)**adenosine**)

ACCESSION NUMBER: 84025253 MEDLINE
DOCUMENT NUMBER: 84025253 PubMed ID: 6892270
TITLE: Differential inhibition by **adenosine** or by
prostaglandin E1 of human platelet aggregation induced by
adenosine 5'-O-(1-thiodiphosphate) and
adenosine 5'-O-(2-thiodiphosphate).
AUTHOR: Cusack N J; Hourani S M
SOURCE: BRITISH JOURNAL OF PHARMACOLOGY, (1982 Feb) 75 (2) 257-9.
Journal code: 7502536. ISSN: 0007-1188.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198312
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19831220

- AB Adenosine 5'-diphosphate (ADP) induces human **platelet aggregation** and **inhibits** stimulated adenylate cyclase. **Adenosine** 5'-O-(1-thiodiphosphate) (ADP-alpha-S) and adenosine 5'-O-(2-thiodiphosphate) (ADP-beta-S) act at the ADP receptor and achieve the same maximal rate of human platelet aggregation as each other. Adenosine and prostaglandin E1, which noncompetitively inhibit ADP-induced aggregation by stimulating adenylate cyclase, inhibit aggregation induced by ADP-x-S more than aggregation induced by ADP-beta-S. ADP-x-S, unlike ADP-beta-S and ADP itself, does not inhibit stimulated adenylate cyclase. This suggests that the inhibition of stimulated adenylate cyclase by ADP, although not a cause of aggregation, may be of physiological importance in reducing the effects of endogenous agents such as adenosine and prostaglandins (for example, prostacyclin) to which the platelet may be exposed.
- TI Differential inhibition by **adenosine** or by prostaglandin E1 of human platelet aggregation induced by **adenosine** 5'-O-(1-thiodiphosphate) and **adenosine** 5'-O-(2-thiodiphosphate).
- AB Adenosine 5'-diphosphate (ADP) induces human **platelet**

L9 ANSWER 69 OF 107 MEDLINE DUPLICATE 26

ACCESSION NUMBER: 84025253 MEDLINE
DOCUMENT NUMBER: 84025253 PubMed ID: 6892270
TITLE: Differential inhibition by **adenosine** or by
prostaglandin E1 of human platelet aggregation induced by
adenosine 5'-O-(1-thiodiphosphate) and
adenosine 5'-O-(2-thiodiphosphate).
AUTHOR: Cusack N J; Hourani S M
SOURCE: BRITISH JOURNAL OF PHARMACOLOGY, (1982 Feb) 75 (2) 257-9.
Journal code: 7502536. ISSN: 0007-1188.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198312
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Adenosine 5'-O-(1-thiodiphosphate) (ADP-alpha-S) and adenosine
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Adenosine and prostaglandin E1, which noncompetitively inhibit ADP-induced
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by ADP-x-S more than aggregation induced by ADP-beta-S. ADP-x-S, unlike
ADP-beta-S and ADP itself, does not inhibit stimulated adenylate cyclase.
This suggests that the inhibition of stimulated adenylate cyclase by ADP,
although not a cause of aggregation, may be of physiological importance in
reducing the effects of endogenous agents such as adenosine and
prostaglandins (for example, prostacyclin) to which the platelet may be
exposed.
TI Differential inhibition by **adenosine** or by prostaglandin E1 of
human platelet aggregation induced by **adenosine**
5'-O-(1-thiodiphosphate) and **adenosine** 5'-O-(2-thiodiphosphate).
AB Adenosine 5'-diphosphate (ADP) induces human **platelet**

(FILE 'HOME' ENTERED AT 10:04:02 ON 07 JAN 2003)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, CAPLUS, USPATFULL' ENTERED AT
10:04:31 ON 07 JAN 2003

L1	1 S ADENOSINE (A) BIND? (A) (GLYCOPROTEIN? OR GPIIBIIIA)
L2	70 S ADENOSINE (3A) (ANTITHROMB? OR (INHIBIT? THROMB))
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L8	203 S L3 AND ADENOSINE/TI
L9	107 DUP REM L8 (96 DUPLICATES REMOVED)

=>

STIC-ILL

YMC
PC681.A1 A57137

From: Gabel, Gailene
Sent: Tuesday, January 07, 2003 10:57 AM
To: STIC-ILL
Subject: 09/853,524

Please provide a copy of the ff. literature ASAP:

- 1) Born and Cross, J. Physiol., 1963, 166:29-30.
- 2) Kitakaze et al., Endogenous adenosine inhibits platelet aggregation during myocardial ischemia in dogs. CIRCULATION RESEARCH, (1991 Nov) 69 (5) 1402-8.
- 3) Soederbaeck et al., Anti-aggregatory effects of physiological concentrations of adenosine in human whole blood as assessed by filtragometry. Clinical Science (1991), 81(5), 691-4.
- 4) Singh et al., Effect of adenosine and inosine administration on platelet function in rabbits. Indian Journal of Physiology and Pharmacology (1990),34(1), 63-4.
- 5) Cusack et al., Differential inhibition by adenosine or by prostaglandin E1 of human platelet aggregation induced by adenosine 5'-O-(1-thiodiphosphate) and adenosine 5'-O-(2-thiodiphosphate). BRITISH JOURNAL OF PHARMACOLOGY, (1982 Feb) 75 (2) 257-9.
- 6) Caen et al., Adenosine inhibition of human platelet aggregation by ADP. NATURE. NEW BIOLOGY, (1972 Oct 18) 239 (94) 211-3.
- 7) Born et al., Role of the competition in inhibition of platelet aggregation by adenosine. ACTA MEDICA SCANDINAVICA. SUPPLEMENTUM, (1971) 525 173-4.

Thanks a bunch,
Gailene R. Gabel
7B15
305-0807

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Mm

From: Gabel, Gailene
Sent: Monday, January 06, 2003 5:03 PM
To: STIC-ILL
Subject: 09/853,524

Please provide a copy of the following literature ASAP:

- 1) Rubin et al., New RGD analogue inhibits human platelet adhesion and aggregation and eliminates platelet deposition on canine vascular grafts. Journal of Vascular Surgery, (1992) 15/4 (683-692).
- 2) Armstrong et al., Inhibition of red blood cell-induced platelet aggregation in whole blood by a nonionic surfactant, poloxamer 188 (RheothRx injection), THROMBOSIS RESEARCH, (1995 Sep 15) 79 (5-6) 437-50.
- 3) ELY et al., PROTECTIVE EFFECTS OF ADENOSINE IN MYOCARDIAL-ISCHEMIA, CIRCULATION, (MAR 1992) Vol. 85, No. 3, pp. 893-904.
- 4) Bastida et al., Differentiation of platelet-aggregating effects of human tumor cell lines based on inhibition studies with apyrase, hirudin, and phospholipase. Cancer Research, (1982) 42/11 (4348-4352).
- 5) Lee et al., In vitro platelet abnormality in adenosine deaminase deficiency and severe combined immunodeficiency. BLOOD, (1979 Mar) 53 (3) 465-71.
- 6) Cattaneo et al., Released adenosine diphosphate stabilizes thrombin-induced human platelet aggregates. BLOOD, (1990 Mar 1) 75 (5) 1081-6.
- 7) Wang et al., Exogenous adenosine application inhibits thrombus formation in stenosed canine coronary artery and partially protects against renewal of thrombus formation by epinephrine. FASEB Journal, (1995) Vol. 9, No. 3, pp. A322.

Thanks a bunch,
Gailene R. Gabel
7B15
305-0807

L9 ANSWER 44 OF 107 MEDLINE DUPLICATE 15

ACCESSION NUMBER: 92035426 MEDLINE
DOCUMENT NUMBER: 92035426 PubMed ID: 1657446
TITLE: Endogenous **adenosine inhibits platelet aggregation** during myocardial ischemia in dogs.
AUTHOR: Kitakaze M; Hori M; Sato H; Takashima S; Inoue M; Kitabatake A; Kamada T
CORPORATE SOURCE: First Department of Medicine, Osaka University School of Medicine, Japan.
SOURCE: CIRCULATION RESEARCH, (1991 Nov) 69 (5) 1402-8.
Journal code: 0047103. ISSN: 0009-7330.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199111
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911127

AB The goal of this study was to clarify that blockade of adenosine receptors during myocardial ischemia causes further reductions in coronary blood flow due to platelet aggregation. Coronary perfusion pressure in 47 open-chest dogs was reduced such that coronary blood flow decreased to one fifth of the control value; thereafter, coronary perfusion pressure was maintained at the low levels. During hypoperfusion, coronary flow was kept low but constant with a massive release of adenosine. When 8-phenyltheophylline, an adenosine receptor antagonist, was infused during coronary hypoperfusion, coronary blood flow (18 +/- 2 ml/100 g/min) gradually decreased at 5-10 minutes of ischemia and reached almost zero at 20 minutes. Three minutes after the onset of ischemia, before further reduction of coronary flow, the microscopic examination revealed the existence of thromboembolization in the small coronary arteries, and the number of platelets in the regional coronary venous blood were significantly decreased, indicating that a further reduction of coronary flow due to treatment with 8-phenyltheophylline is attributed to thromboembolism caused by platelet aggregations. This reduction of coronary flow and formation of thromboembolism were inhibited by the treatments with dibutyryl cAMP, forskolin, and yohimbine, indicating that this thromboembolization during a lack of adenosine activity is due to platelet aggregation and that platelet aggregation caused by 8-phenyltheophylline is triggered by stimulation of alpha 2-adrenoceptors by released norepinephrine during ischemia. We demonstrate that adenosine, generated endogenously in response to ischemia, **inhibits platelet aggregation**. The finding that **adenosine** is not merely a vasodilator but that it also regulates thrombosis has major implications for designing new strategies of myocardial salvage.

TI Endogenous **adenosine inhibits platelet aggregation** during myocardial ischemia in dogs.

L22 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:193637 BIOSIS
DOCUMENT NUMBER: PREV199598207937
TITLE: **Exogenous adenosine** application
inhibits thrombus formation in stenosed canine coronary
artery and partially protects against renewal of thrombus
formation by epinephrine.
AUTHOR(S): Wang, T.; Lavis, J.; Bakalyar, D. M.; Catlin, T. R.;
Timmis, G. C.; O'Neill, W. W.
CORPORATE SOURCE: William Beaumont Hosp., Royal Oak, MI 48073 USA
SOURCE: FASEB Journal, (1995) Vol. 9, No. 3, pp. A322.
Meeting Info.: Experimental Biology 95, Part I Atlanta,
Georgia, USA April 9-13, 1995
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English
TI **Exogenous adenosine** application inhibits thrombus
formation in stenosed canine coronary artery and partially protects
against renewal of thrombus formation by epinephrine.
IT Miscellaneous Descriptors
ADENOSINE; ANTICOAGULANT-DRUG; ARTERIAL **THROMBOSIS**;
CYCLIC FLOW REDUCTION; LEFT CIRCUMFLEX CORONARY ARTERY; MEETING
ABSTRACT; STENOSIS

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30-

From: Gabel, Gailene
Sent: Tuesday, January 07, 2003 10:57 AM
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Please provide a copy of the ff. literature ASAP:

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- 2) Kitakaze et al., Endogenous adenosine inhibits platelet aggregation during myocardial ischemia in dogs. CIRCULATION RESEARCH, (1991 Nov) 69 (5) 1402-8.
- 3) Soederbaeck et al., Anti-aggregatory effects of physiological concentrations of adenosine in human whole blood as assessed by filragnetometry. Clinical Science (1991), 81(5), 691-4.
- 4) Singh et al., Effect of adenosine and inosine administration on platelet function in rabbits. Indian Journal of Physiology and Pharmacology (1990),34(1), 63-4.
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- 6) Caen et al., Adenosine inhibition of human platelet aggregation by ADP. NATURE. NEW BIOLOGY, (1972 Oct 18) 239 (94) 211-3.
- 7) Born et al., Role of the competition in inhibition of platelet aggregation by adenosine. ACTA MEDICA SCANDINAVICA. SUPPLEMENTUM, (1971) 525 173-4.

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Authors	Soderback_U Sollevi_A Wallen_N_H Larsson_P_T Hjemdahl_P
Journal title	Clinical Science
ISSN	0143-5221
Publisher	Portland Press
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Supplement	0
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Anti-aggregatory effects of physiological concentrations of adenosine in human whole blood as assessed by filtragometry

U. SÖDERBÄCK¹, A. SOLLEVI^{1,2}, N. H. WALLEN^{1,3}, P. T. LARSSON¹ AND P. HJEMDAHL^{1,3}

¹Department of Pharmacology, Karolinska Institute, Stockholm, Sweden, and Departments of ²Anaesthesiology and ³Clinical Pharmacology, Karolinska Hospital, Stockholm, Sweden

(Received 10 May 1991; accepted 20 June 1991)

SUMMARY

1. The anti-aggregatory effect of adenosine (0.3–10 $\mu\text{mol/l}$), alone or in combination with the adenosine-uptake inhibitor dipyridamole (2 $\mu\text{mol/l}$), was studied *in vitro* in whole blood from 11 healthy subjects by filtragometry.

2. ADP (0.05–0.1 $\mu\text{mol/l}$) was used to reduce the filter occlusion time (t_A , a measure of platelet aggregate formation in blood) from approximately 600 s to 71–101 s in the absence of other agents.

3. Adenosine was infused into the tubing system of the filtragometer, yielding a contact time of ≈ 25 s with the blood before the filter. Adenosine did not influence the aggregatory response to ADP significantly at 0.3 $\mu\text{mol/l}$ in plasma, whereas t_A was prolonged by $19 \pm 6\%$ ($P < 0.02$) at 1 $\mu\text{mol/l}$ adenosine and by $259 \pm 78\%$ ($P < 0.02$) at 3 $\mu\text{mol/l}$ adenosine.

4. When the rapid elimination of adenosine from plasma was prevented by 2 $\mu\text{mol/l}$ dipyridamole, adenosine caused marked prolongation of ADP-induced t_A , with significant effects at 0.3 $\mu\text{mol/l}$ ($+143 \pm 72\%$, $P < 0.05$). Dipyridamole *per se* did not affect t_A values.

5. The present results suggest that adenosine has a transient anti-aggregatory effect in whole blood at about 0.3 $\mu\text{mol/l}$, as this is the highest possible calculated concentration of adenosine at the filter of the apparatus when 1 $\mu\text{mol/l}$ adenosine is infused in the absence of dipyridamole or when 0.3 $\mu\text{mol/l}$ adenosine is infused in its presence.

6. It is concluded that adenosine has anti-aggregatory effects at submicromolar (physiological) concentrations in human whole blood. The effect of adenosine seems to be transient, indicating a role for adenosine as a localized platelet-stabilizing factor in the vicinity of, for example, the endothelium.

Key words: adenosine, dipyridamole, platelet aggregation, whole blood.

INTRODUCTION

Platelet aggregation is inhibited by adenosine [1] via enhanced accumulation of cyclic AMP [2]. Adenosine stimulates adenylate cyclase through specific cell-surface receptors of the A_2 -type [3, 4]. Adenosine has an extremely short (≤ 10 s) half-life in plasma [5], primarily due to cellular uptake. The basal free plasma concentration of adenosine in normal human blood has varied when measured in different studies, but is in the range 0.02–0.3 $\mu\text{mol/l}$ [6–8]. Adenosine levels in plasma may be increased in micromolar concentrations during various physiological or pathophysiological conditions [8, 9]. Due to the rapid elimination of adenosine in blood during incubation *in vitro*, it has been difficult to demonstrate anti-platelet effects of adenosine at physiological concentrations. With whole-blood impedance aggregometry, adenosine inhibits aggregation at 1–5 $\mu\text{mol/l}$ [10]. With filtragometry [11], which measures the presence of platelet aggregates in continuously drawn blood, it might be possible to study the influence of labile anti-aggregatory compounds by infusing them into the tubing system through which the blood is drawn.

The aim of this study was thus to determine whether physiological concentrations of adenosine inhibit platelet aggregation in whole blood by using the filtragometry technique. For these studies aggregation was enhanced by ADP. Furthermore, the effects of dipyridamole, which prevents the elimination (due to inhibition of uptake) of adenosine from plasma [12], were studied in the presence and absence of exogenous adenosine.

MATERIALS AND METHODS

Blood from 11 healthy male subjects, who had not taken any acetylsalicylic acid during the preceding 14 days and

Correspondence: Dr Alf Sollevi, Department of Anaesthesiology, Karolinska Hospital, PO Box 60 500, S-104 01 Stockholm, Sweden.

who had refrained from methylxanthine-containing beverages for 12 h, was studied. The protocol was approved by the Ethics Committee of the Karolinska Institute.

Blood (250–300 ml) from an antecubital vein was drawn into siliconized syringes with 1/10 v/v saline (154 mmol/l NaCl) containing low-*M*, heparin (Fragmin; Kabi, Stockholm, Sweden; final concentration 10 i.u./ml of blood). The blood was stored in a plastic bag and was continuously mixed on a rocker platform. Platelet counts were unaffected by 6 h storage $[(181 \pm 17) - (183 \pm 18) \times 10^9/l]$.

The filtragometry technique [11, 13] uses a nickel filter with 20 μm pores and measures the time in seconds for platelet aggregates to partly occlude the filter (t_A). Pressure transducers record the pressure differential (ΔP) across the filter at 37°C. As platelet aggregates gradually occlude the filter, ΔP is raised. The perfusion pressure difference is recorded on a strip-chart recorder. When the filter is occluded to approximately 25% ($=\Delta P$ corresponding to 5 mmHg) the t_A is determined. The plastic tubing system was filled with 3.8% (w/v) sodium citrate which was filtered and degassed before use in order to remove particles and air bubbles in the system. For each measurement 20 ml of blood was transferred to a plastic vial and was continuously stirred with a Teflon stirring bar. Blood was drawn from the vial with a motor-driven syringe (flow rate 2 ml/min). To enhance aggregation, ADP (final concentration in plasma 0.05–0.1 $\mu\text{mol/l}$) was infused at the beginning of the tubing system. Adenosine (final concentration in plasma 0.3–10 $\mu\text{mol/l}$) was also infused at the beginning of the tubing system at a flow rate of 0.08 ml/min. The transit time from the drug infusion site to the filter was approximately 25 s. The tubing system and all equipment in contact with blood were siliconized. Dipyridamole experiments were performed by preincubating the blood in the vial for 2 min with continuous stirring. Saline infusion was the reference for unstimulated blood. ADP challenge without other drug treatment was intermittently performed throughout the experiment as control. The influence of anti-aggregatory drugs was studied by comparison with t_A values during ADP controls run before or after the treatment

investigated, and was expressed as percentage change. Filtragometry data are expressed as t_A in s.

Because of the asymmetrical distribution of t_A data, logarithmic transformation was performed [11]. Statistical differences were evaluated by Wilcoxon's signed rank test. A *P* value of less than 0.05 was considered significant. Data are shown as means \pm SEM or as percentage change from control.

Adenosine was obtained from Calbiochem (La Jolla, CA, U.S.A.). ADP was purchased from Sigma (St Louis, MO, U.S.A.). Dipyridamole was supplied by Boehringer (Ingelheim, Germany). Fragmin was a kind gift from Kabi Vitrum AB (Stockholm, Sweden). The drugs were dissolved in saline.

RESULTS (FIG. 1 AND TABLE 1)

The t_A in unstimulated blood (saline infusion) ranged from 398 s to >682 s, with a mean (\pm SEM) value of 567 ± 35 s. ADP (0.05–0.1 $\mu\text{mol/l}$) decreased t_A to

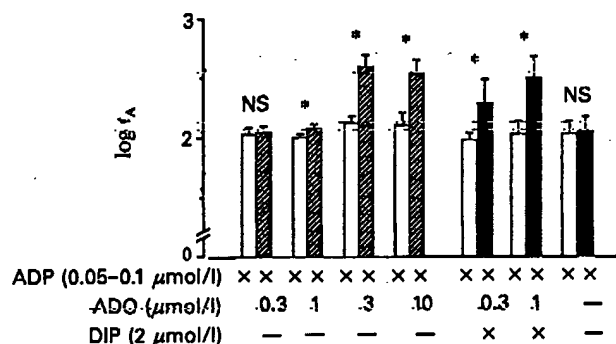


Fig. 1. t_A measured by filtragometry during ADP stimulation (0.05–0.1 $\mu\text{mol/l}$) and the influence of adenosine (0.3–10 $\mu\text{mol/l}$) or adenosine plus dipyridamole (2 $\mu\text{mol/l}$), expressed as the logarithm of t_A . Values are means \pm SEM. Untransformed values and percentage changes are shown in Table 1, as are the numbers of observations. Significance values represent comparisons with corresponding ADP controls. Abbreviations: ADO, adenosine; DIP, dipyridamole; NS, not significant.

Table 1. Effect of adenosine and adenosine plus dipyridamole on ADP-induced aggregation in human whole blood, as assayed by filtragometry *in vitro*

t_A , the percentage change in t_A compared with the ADP control, the numbers of observations and the significance levels are shown. Values are means \pm SEM. Abbreviations: ADO, adenosine; DIP, dipyridamole.

	<i>n</i>	t_A (s)	Change in t_A from ADP control (%)	<i>P</i> value
0.3 $\mu\text{mol/l}$ ADO	6	114 \pm 16	3 \pm 10	> 0.8
1 $\mu\text{mol/l}$ ADO	11	122 \pm 11	19 \pm 6	< 0.02
3 $\mu\text{mol/l}$ ADO	7	442 \pm 76	259 \pm 78	< 0.02
10 $\mu\text{mol/l}$ ADO	5	384 \pm 90	210 \pm 81	< 0.05
0.3 $\mu\text{mol/l}$ ADO + 2 $\mu\text{mol/l}$ DIP	5	284 \pm 119	143 \pm 72	< 0.05
1 $\mu\text{mol/l}$ ADO + 2 $\mu\text{mol/l}$ DIP	5	421 \pm 128	240 \pm 96	< 0.05
2 $\mu\text{mol/l}$ DIP	5	133 \pm 44	7 \pm 6	> 0.2

96 ± 6 s (range 71–101 s) at the start, and to 131 ± 16 s (range 68–238 s) at the end of the experiments. ADP-stimulated t_A values were not significantly influenced by $0.3 \mu\text{mol/l}$ adenosine. At $1 \mu\text{mol/l}$ in plasma at the site of infusion, adenosine prolonged t_A by approximately 20%, and this prolongation was further increased (to above 200%) by higher adenosine concentrations. Preincubation of the blood with dipyridamole ($2 \mu\text{mol/l}$) had no influence *per se* on ADP-stimulated t_A values. An even higher dipyridamole concentration ($13.3 \mu\text{mol/l}$) was used in one subject without affecting ADP-stimulated t_A values (101 s versus 103 s). In the presence of dipyridamole, adenosine antagonized ADP-induced aggregability at $0.3 \mu\text{mol/l}$, producing a $143 \pm 72\%$ ($P < 0.05$) prolongation of t_A . This effect of adenosine was further enhanced at $1 \mu\text{mol/l}$ in the presence of dipyridamole ($+240 \pm 96\%$, $P < 0.05$).

DISCUSSION

Filtragometry [11] proved to be a sensitive method for studies of platelet aggregability *in vitro*, as ADP enhanced aggregation (i.e. shortened t_A markedly) at concentrations of 0.05 – $0.1 \mu\text{mol/l}$. Conventional techniques *in vitro* require micromolar concentrations of ADP, whether in whole blood using impedance methodology [14] or in platelet-rich plasma using the turbidimetric technique [1]. The specificity of filtragometry as a measure of platelet aggregation has been evaluated previously. Using scanning electron microscopy to visualize the filter, it is evident that filter occlusion is mainly due to platelet aggregates; only a few trapped erythrocytes are observed. Aggregated or entrapped leucocytes are not seen on the filter [11]. Work in our laboratory (N. H. Wallen *et al.*, unpublished work), using the filtragometer *in vitro*, show no significant difference in the EC_{50} for ADP between whole blood and platelet-rich plasma (which does not contain leucocytes or erythrocytes). This further supports the concept that the filtragometer really measures platelet aggregates and that the contribution of leucocytes and erythrocytes to filter occlusion are of minor importance. Filtragometry revealed that adenosine inhibited the ADP-induced platelet aggregate formation in whole blood at a plasma concentration of $1 \mu\text{mol/l}$ without and at $0.3 \mu\text{mol/l}$ with the adenosine-uptake inhibitor dipyridamole. The latter drug had no effect *per se*. Adenosine is rapidly removed from plasma, mainly by active uptake into erythrocytes. Enzymic removal by adenosine deaminase seems to be of importance only at high substrate concentrations [15]. The half-life of adenosine in human undiluted whole blood is less than 10 s at micromolar concentrations of the compound [5]. The elimination of low adenosine concentrations (0.1 – $1 \mu\text{mol/l}$) may be even more rapid in blood [8]. As the estimated contact time for adenosine with the blood before it reaches the filter of the filtragometer is approximately 25 s, the adenosine concentration is reduced to less than $0.3 \mu\text{mol/l}$ in the vicinity of the filter when $1 \mu\text{mol/l}$ is infused. Adenosine at a concentration of $0.3 \mu\text{mol/l}$ had no effect *per se*, but was effective in the presence of inhibition of adenosine uptake

by dipyridamole. Our data therefore indicate that sub-micromolar concentrations of adenosine have anti-aggregatory effects in human whole blood.

It may be hypothesized that the effect of adenosine is short-lasting and that it is the adenosine concentration near the filter which was the relevant one. The effective concentration of $1 \mu\text{mol/l}$ added adenosine would be less than $0.3 \mu\text{mol/l}$ close to the filter in the absence of dipyridamole and approximately the same as at the site of infusion in its presence, since adenosine deaminase is of minor importance compared with elimination by uptake [15]. Since adenosine was more than three-fold more potent in the presence of dipyridamole and the contact time was short, our data indicate that the anti-aggregatory effect of the compound is dependent on its continuous presence. Thus a short-lasting elevation of the adenosine concentration to $0.3 \mu\text{mol/l}$ in the blood upstream could not maintain the anti-aggregatory response at the filter, suggesting that brief activation of adenosine receptors also causes short-lasting anti-aggregatory effects. Dipyridamole does not have platelet-inhibiting effects mediated by phosphodiesterase inhibition, as anti-aggregatory responses to high dipyridamole concentrations ($50 \mu\text{mol/l}$) in whole blood are blocked by treatment with adenosine deaminase [16]. The anti-aggregatory response to dipyridamole has indeed been attributed to raised plasma adenosine concentrations in other assay systems [10, 17]. In agreement with these findings, we found no effect of dipyridamole *per se*. The concentration mainly used by us ($2 \mu\text{mol/l}$) is clinically relevant [12] and is effective for uptake inhibition in human blood [8].

How might adenosine then influence platelet function *in vivo*? The present results, showing transient anti-aggregatory effects at physiological concentrations of adenosine, suggest that enhanced adenosine release from endothelial cells, during for example hypoxia [18], would produce a local effect. Such a modulating effect of adenosine may be an additional mechanism in the regional control of platelet function and haemostasis.

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REFERENCES

1. Born, G.V.R. & Cross, M.J. The aggregation of blood platelets. *J. Physiol. (London)* 1963; 168, 178–95.
2. Haslam, R.J. & Cusack, N.J. Blood platelet receptors for ADP and for adenosine. In: Burnstock, G., ed. *Purinergic receptors: receptors and recognition*. Series B. London: Chapman and Hall, vol. 12, 221–85.
3. Ukena, D., Poeschla, E., Hutterman, E. & Schwabe, U. Effects of *N*-ethylmaleimide on adenosine receptors of rat fat cells and human platelets. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1984; 327, 247–53.

4. Schwabe, U. Use of radioligands in the identification, classification, and study of adenosine receptors. *Methods Pharmacol.* 1985; **6**, 239-78.
5. Klabunde, R.E. Dipyridamole inhibition of adenosine metabolism in human blood. *Eur. J. Pharmacol.* 1983; **93**, 21-6.
6. Sollevi, A., Östergren, J., Fagrell, B. & Hjemdahl, P. Theophylline antagonizes cardiovascular responses to dipyridamole in man without affecting increases in plasma adenosine. *Acta Physiol. Scand.* 1984; **121**, 165-71.
7. Ontyd, J. & Schrader, J. Measurement of adenosine, inosine, and hypoxanthine in human plasma. *J. Chromatogr.* 1984; **307**, 404-9.
8. Möser, G.H., Schrader, J. & Deussen, A. Turnover of adenosine in plasma of human and dog blood. *Am. J. Physiol.* 1989; **256** (Cell Physiol. **25**), C799-806.
9. Sollevi, A. Cardiovascular effects of adenosine in man; possible clinical implications. *Prog. Neurobiol.* 1986; **27**, 319-49.
10. Söderbäck, U. & Sollevi, A. Effect of dipyridamole and a dipyridamole-like compound (R-E 244) on aggregation and cyclic AMP accumulation in human platelets. *Thromb. Res.* (In press).
11. Hornstra, G. & ten Hoor, F. The filtragometer: a new device for measuring platelet aggregation in venous blood in man. *Thromb. Diath. Haemorrh.* 1975; **34**, 531-44.
12. Pedersen, A.K. Specific determination of dipyridamole in serum by high-performance liquid chromatography. *J. Chromatography* 1979; **162**, 90-103.
13. Larsson, P.T., Hjemdahl, P., Olsson, G., Egberg, N. & Hornstra, G. Altered platelet function during mental stress and adrenaline infusion in humans: evidence for an increased aggregability *in vivo* as measured by filtragometry. *Clin. Sci.* 1989; **76**, 369-76.
14. Cardinal, D.C. & Flower, R.J. The electronic aggregometer: a novel device for assessing platelet behavior in blood. *J. Pharmacol. Methods* 1980; **3**, 135-58.
15. Arch, J.R.S. & Newsholme, E.A. The control of the metabolism and the hormonal role of adenosine. In: Campbell, P.W. & Aldridge, W.N., eds. *Assays in biochemistry*. London: Academic Press, vol. 14, 82-123.
16. Gresele, P., Arnout, J., Deckmyn, H. & Vermynen, J. Mechanism of the antiplatelet action of dipyridamole in whole blood: modulation of adenosine concentration and activity. *Thromb. Haemostasis* 1986; **55**, 12-18.
17. Edlund, A., Sidén, A. & Sollevi, A. Evidence for an anti-aggregatory effect of adenosine at physiological concentrations and its role in the action of dipyridamole. *Thromb. Res.* 1987; **45**, 183-90.
18. Nees, S., Gerbes, A.L., Willershausen-Zönnchen, B. & Gerlach, E. Purine metabolism in cultured coronary endothelial cells. *Adv. Exp. Med. Biol.* 1979; **122**, 25-30.